British Journal of Pharmacology (1998) 124, 190–196
http://www.stockton-press.co.uk/bjp

Ovalbumin-induced neurogenic inflammation in the bladder of sensitized rats

[1]Amrita Ahluwalia, [2,3]Sandro Giuliani, [1]Ramona Scotland & [2]Carlo Alberto Maggi

[1]Centre for Clinical Pharmacology, The Rayne Institute, University College London, 5 University St, London WC1E 6JJ and [2]Menarini Ricerche, Department of Pharmacology, Florence, Italy

1 We have developed and characterized a model of immediate hypersensitivity/inflammation of the urinary bladder *in vivo* induced by local application of ovalbumin (OA) in OA- sensitive female rats. Two parameters of the inflammatory response were assessed following OA challenge: plasma protein extravasation (PPE) and changes in smooth muscle reactivity. The former was estimated by measurement of Evans blue extravasation at 0.5, 2, 4, 8 and 24 h time point following *in vivo* challenge. Changes in reactivity were determined by measurement of isotonic tension responses of urinary bladder strips following OA challenge *in vitro*.

2 Acute *in vivo* intravesical OA challenge (10 mg in 0.3 ml saline) in actively sensitized female Wistar rats caused a time-dependent PPE in the urinary bladder which was biphasic with peak responses at 2–4 and 24 h.

3 The PPE response to acute OA challenge, above base-line, at 2 h was abolished by systemic capsaicin pretreatment (50 mg kg^{-1}, s.c., 4 days before use) ($P<0.05$) whilst the response at 24 h was unaffected. The 2 h time point was then used for further studies.

4 Degranulation of mast cells, achieved by pretreatment with compound 48/80 (5 mg kg^{-1}, s.c. for 3 consecutive days), completely abolished the PPE response to OA challenge at the 2 h time point.

5 The tachykinin NK_1 receptor antagonist, SR 140333 (0.1 μmol kg^{-1}, i.v.), abolished the 2 h PPE response whilst the tachykinin NK_2 receptor antagonist MEN 11420 (0.1 μmol kg^{-1}, i.v.) appeared to reduce the response by approximately 50% but this did not reach significance. The bradykinin B_2 receptor antagonist, Hoe 140 (0.1 μmol kg^{-1}, i.v.), similarly to SR 140333, blocked the 2 h PPE response to OA, whereas the selective B_1 receptor antagonist B 9858 (0.1 μmol kg^{-1}, i.v.) had no significant effect. Inhibition of cyclo-oxygenase (COX) achieved by pretreatment with the COX inhibitor dexketoprofen (5.3 μmol kg^{-1}, i.v.) also blocked the PPE response, whilst the leukotriene receptor antagonist ONO 1078 (1 μmol kg^{-1}, i.v.) significantly reduced PPE by about 80%.

6 In the rat isolated urinary bladder OA (1 mg ml^{-1}) challenge produced a biphasic response with a rapidly achieved maximal contraction followed by a sustained contraction for approximately 25 min. *In vitro* capsaicin pretreatment (10 μM for 15 min) significantly attenuated the duration of the sustained contraction whilst having no effect on the maximum contractile response achieved. *In vivo* pretreatment of animals with compound 48/80 significantly attenuated (42%) the maximum contractile response. Combination of both treatments almost completely abolished the response. *In vitro* treatment with Hoe 140 (1 μM) had no significant effect on the response to OA and neither did ONO 1078 (1 μM).

7 These results show that both the early inflammatory response and alterations in smooth muscle reactivity to OA challenge in actively sensitized animals are dependent on mast cell degranulation and the activation of sensory C-fibres. Furthermore this model of allergic cystitis may be useful for investigating both the processes involved and potential novel therapies in the treatment of interstitial cystitis.

Keywords: Ovalbumin-sensitization; urinary bladder; plasma protein extravasation; allergic reaction; interstitial cystitis

Introduction

The aetiological basis for the chronic inflammatory disease of interstitial cystitis is unclear whereas the symptoms themselves (severe urinary urgency, frequency, nocturia and pain) are well-defined. It has been proposed that interstitial cystitis may develop in response to an immune reaction and, in support of this, immunoglobulin and complement deposits have been identified in affected bladders. Furthermore there have been several accounts of systemic allergic disease, such as asthma and rheumatoid arthritis, associated with interstitial cystitis (Hellstrom *et al*., 1979). Other examples suggest that interstitial cystitis may be a consequence of local allergy developing in response to certain allergenic stimuli such as silk (Hollander, 1994).

[3]Author for correspondence at: Department of Pharmacology, Menarini Ricerche, Via Rismondo 12A, 50131 Florence, Italy.

Models attempting to mimic immunologically-induced bladder inflammation have therefore been developed. Some studies have shown that systemic sensitization with a specific antigen, namely ovalbumin, followed by acute systemic challenge produces an inflammatory response. Evans *et al*. (1988) showed that acute challenge with ovalbumin of sensitized guinea-pigs causes plasma extravasation in several tissues including the bladder. Further studies demonstrated that intravesical administration of OA in sensitized guinea-pigs produces a local inflammatory response associated with increased frequency of micturition, reduced pressure and volume threshold for micturition (Christensen *et al*., 1990; Kim *et al*., 1991). These characteristics are all reminiscent of the symptoms of interstitial cystitis.

Acute hypersensitivity responses are characterized by plasma protein extravasation, white cell accumulation and increased blood flow. The immediate response is dependent on

the degranulation of mast cells following interaction of the antigen with IgE bound to the mast cell. Several studies show that the aforementioned responses are sequelae following mast cell degranulation (Galli, 1993). Indeed interstitial cystitis seems to be characterized by an increased number of mast cells present in the bladder wall and this mastocytosis has been frequently used as a diagnostic tool for the prognosis of interstitial cystitis (Messing & Stamey, 1978). Additionally, another feature of the allergic response is the accumulation of eosinophils, a prominent feature of the late-response (Hogan & Foster, 1996). There are studies demonstrating that cystitis is associated with an eosinophil infiltrate, further supporting the hypothesis that interstitial cystitis may have an immunological basis (Hellstrom *et al*., 1979).

Models which mimic such immunologically-derived responses would be of potential value in further elucidation of the mechanisms involved in the development of interstitial cystitis, as well as for the development of drug treatment candidates for the treatment of interstitial cystitis. Based upon this premise we have developed a model of immune-complex induced bladder inflammation.

Methods

Sensitization

Female Wistar rats (150–175 g) were purchased from Charles River, Italy. Sensitization of animals was achieved by intraperitoneal injection of a mixture of 1 mg OA and 100 mg aluminium hydroxide suspended in 1 ml of saline. Fourteen days later rats were anaesthetized with pentobarbitone (45 mg kg^{-1}, i.p.) for intravesical administration of OA. The urinary bladder was manually emptied of urine and then catheterized via the urethra by means of a polyethylene tube (PE 50, Clay Adams) inserted to 2 cm from the external urethral orifice. The animals received 0.3 ml of OA (10 mg OA in sterile saline) or the vehicle control (saline) over 10 s and the catheter was allowed to remain in place for a further 10 s before withdrawal.

Plasma protein extravasation

Plasma protein extravasation (PPE) in the bladder, following OA challenge, was measured by the Evans blue technique (Saria & Lundberg, 1983) in sensitized and non-sensitized female rats (210–250 g). PPE was measured at various times after OA intravesical application over a period spanning from 0.5 to 24 h. Rats were anaesthetized with pentobarbitone (45 mg kg^{-1}, i.p.) for both cannulation of the left jugular vein (PE 50 polyethylene tubing) for i.v. administration and for intravesical OA challenge. Intravesical instillation were performed at 0.5, 2, 4, 8 and 24 h before Evans blue procedure. For 4, 8 and 24 h time points a further administration of the anaesthetic was necessary. Evans blue (50 mg kg^{-1}, i.v.) was administered and 5 min later the dye flushed out of the cardiovascular system by perfusion with warm (37°C) saline (25 ml min^{-1} for 2 min) via intracardiac puncture: the right atria were incised to allow the expulsion of the perfusion medium. The end point of each experiment was considered the starting of the intracardiac perfusion procedure. The urinary bladder was then excised, the extraneous tissue removed, washed in saline and blotted dry before weighing. The Evans blue content was determined by extraction of the Evans blue from each bladder in 100% formamide (4 ml) at 60°C for 24 h. The dye content was determined by the measurement of absorbance at 620 nm with an automated microplate reader EL 312 (Bio-Tek Instruments, U.S.A.). The amount of dye extravasated in each bladder was determined from an Evans blue standard curve and expressed as ng mg^{-1} of tissue weight.

To ascertain the involvement of activation of sensory nerves in the development of plasma extravasation in this system, animals were pretreated with a single subcutaneous injection of capsaicin (50 mg kg^{-1}) 4 days before the acute OA challenge. This capsaicin treatment produces >90% depletion of the sensory neuropeptides content in the urinary bladder (Maggi *et al*., 1988). The effect of this treatment on the response to OA over the 24 h time course was investigated. From this curve, time points sensitive to capsaicin treatment were further studied to characterize the part of the plasma extravasation response involving sensory afferent activation. The involvement of mast cells in these responses was also determined by chronic mast cell degranulation with 3 consecutive daily s.c. injections of compound 48/80 (5 mg kg^{-1}). Animals were used on the 3rd day.

In a second series of experiments the effects of intravenous administration of certain receptor antagonists or enzyme inhibitors on the PPE response at 2 h after OA challenge were assessed and compared with vehicle-treated rats carried out on the same day. The drug treatments employed in this study and their relevant modes of action are described in Table 1, which also cites the references for previous studies verifying the efficacy of the doses of the agents used in this study.

In vitro *experiments*

Female Wistar rats (210–250 g) were killed by cervical dislocation and the whole urinary bladder removed and cleared of extraneous tissue. Longitudinal strips of the bladder (approximately 2–3 mm wide) were mounted in 5 ml organ baths containing Krebs solution oxygenated with 96% O_2 and 4% CO_2 maintained at 37°C under a constant load of 5 mN. The composition of the Krebs solution was as follows (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.5, $CaCl_2$ 2.5, KH_2PO_4 1.2 and glucose 11. The contractile activity of the bladder was recorded with Basile isotonic transducers and displayed on a pen recorder (Unirecord 7050, Basile, Italy). An incubation period of 30 min was allowed before the

Table 1 Different intravenous drug treatments used against ovalbumin-induced plasma protein extravasation in the urinary bladder of the rat *in vivo*

Drug	*Mode of action*	*Pretreatment*	*References*
SR 140333	NK_1 receptor antagonist	0.1 μmol kg^{-1}, 1 h	Emonds-Alt *et al*., (1993)
MEN 11420	NK_2 receptor antagonist	0.1 μmol kg^{-1}, 15 min	Lecci *et al*., (1997)
B 9858	B_1 receptor antagonist	0.1 μmol kg^{-1}, 15 min	Stewart *et al*., (1996)
Hoe 140	B_2 receptor antagonist	0.1 μmol kg^{-1}, 15 min	Maggi *et al*., (1993)
ONO 1078	Leukotriene antagonist	1.0 μmol kg^{-1}, 15 min	Nakagawa *et al*., (1993)
Dexketoprofen	Cyclo-oxygenase inhibitor	5.3 μmol kg^{-1}, 15 min	Mauleon *et al*., (1996)

measurement of the first contractile response to neurokinin A (NKA, 0.1 μM). The preparation was considered fully equilibrated once constant responses to NKA, with 30 min washing intervals between responses, were obtained. Following this the bladder was subjected to OA (1 mg ml^{-1}) and the response measured.

To determine the involvement of capsaicin-sensitive afferent nerves in mediating this response, capsaicin (10 μM) was added to the bathing medium and left in contact with the tissue for 15 min to achieve desensitization of sensory nerves (Maggi, 1995 for a review), after which it was removed by thorough washing. After 30 min, with an interevening wash, OA was administered. To demonstrate the involvement of mast cells on these responses, bladders were taken from animals which had been systemically treated with compound 48/80 as described earlier. The effects of ONO 1078 (1 μM) and Hoe 140 (1 μM), with 15 min contact times, upon the response to OA were also determined.

Drugs

Albumin chicken eggs (ovalbumin, OA), capsaicin and compound 48/80 were purchased from Sigma Chemical Co. (St Louis, U.S.A.). Evans blue was purchased from EGA Chemie (Steinheim, Germany). SR 140333 ((**S**)1-{2-[3-(3,4-dichlorophenyl)-1(3-isopropoxyphenylacetyl) piperidin-3yl]ethyl} -4-phenyl-1-azoniabicyclo [2,2,2]octane chloride) was kindly provided by Drs Emonds-Alt and Le Fur (Sanofi Recherche, Montpellier, France). MEN 11420 (c{[(β-D-GlcNAc)-Asn-Asp-Trp-Phe-Dpr-Leu]c(2β-5β)), Hoe 140 (D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) and B 9858 (Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic) were synthesized in the Chemistry Department of Menarini Ricerche, S(+)-ketoprofen (dexketoprofen) and ONO 1078 (4-oxo-8-[4-(4-phenylbutoxy)-benzoylamino]-2-tetrasol-5-yl)-4H-1-benzopyran hemihydrate) were synthesized at Laboratorios Menarini (Barcelona, Spain).

Statistical analysis

Values are expressed as mean$\pm$s.e.mean. Statistical analysis was performed by means of Student's t test for unpaired data or by means of ANOVA followed by the Bonferroni test. For groups with different standard deviations, the alternate Welch t test was used. Values were considered significantly different when $P<0.05$.

Results

Plasma protein extravasation

Intravesical administration of OA (1 mg in 0.3 ml saline) in OA-sensitized rats caused a time-dependent PPE ($n=11-18$ for each time point) (Figure 1) which was significantly different from that achieved by intravesical administration of saline alone. At 0.5 h the PPE extravasation after OA was not different from control vehicle (Figure 1). The maximum response was achieved between 2 and 4 h and peaked again at 24 h. At 4 h, intravesical instillation of the vehicle induced an inflammatory reaction probably due to catheterization: Evans blue content of the bladder reached 75 ± 17 ng mg^{-1} tissue. At 2 and 8 h, intravesical saline administration in OA-sensitized animals induced a PPE response of 41 ± 6 and 57 ± 9 ng ml^{-1} tissue which was higher than that induced by OA in control unsensitized rats, which gave responses of 25 ± 12 ng mg^{-1} tissue ($n=4$) and 10 ± 5 ng mg^{-1} tissue ($n=4$), respectively, showing a general increased reactivity to intravesical instillation in sensitized animals.

Systemic treatment with capsaicin (50 mg kg^{-1}, 4 days before the experiment) blocked the OA-induced PPE at all time points except the 24 h time point. Figure 2 shows the effect of capsaicin on the response to OA at 2 h ($P<0.05$, $n=16$), as representative of the time points sensitive to capsaicin, and 24 h ($n=14$) as the point that did not respond. To study the neurogenic component of the OA-induced response the 2 h time point was used for all of the ensuing studies.

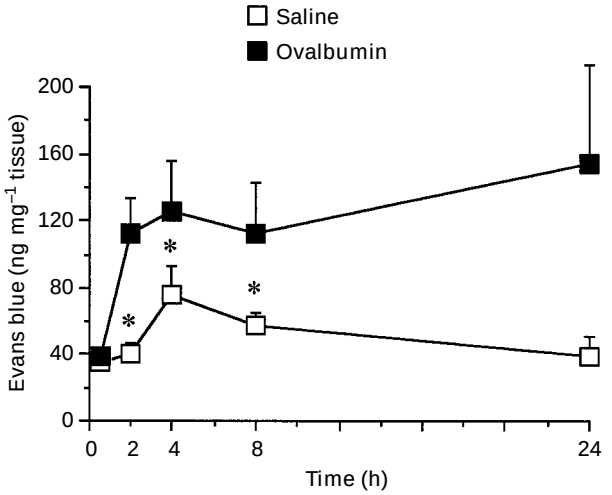

Figure 1 Time-dependent plasma protein extravasation (PPE) induced by acute challenge with intravesical ovalbumin (OA) (10 mg in 0.3 ml saline) or the vehicle in OA-sensitized rats (1 mg OA + 100 mg $A1(OH)_3$ in 1 ml saline, i.p. 14 days before challenge). PPE was tested at various time point from 0.5 to 24 h. Values shown are mean of $n=11-18$ animals; vertical lines indicate s.e.mean. Statistical significance is shown as $*P<0.05$ (OA group compared with vehicle group at each time point).

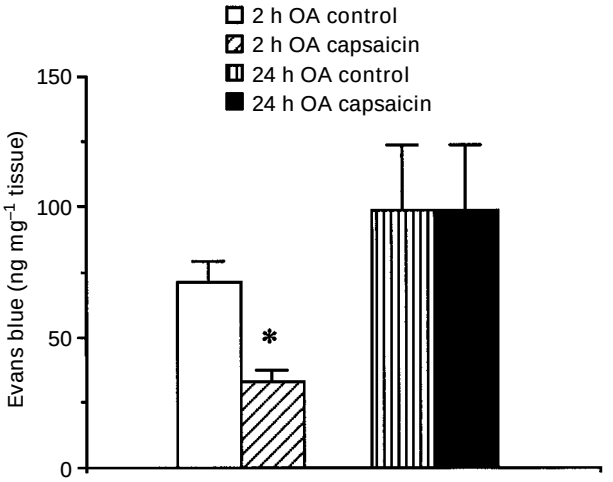

Figure 2 Effect of capsaicin pretreatment (50 mg kg^{-1}, s.c., 4 days before) on the plasma protein extravasation at 2 and 24 h intravesical ovalbumin (OA)-challenge in control untreated animals and in capsaicin-treated animals. Values shown are mean$\pm$s.e.mean of $n=14-16$ animals. Statistical significance is shown as $*P<0.05$.

The 2 h OA response was significantly attenuated in animals pretreated with compound 48/80, giving responses of 29 ± 5 ng mg^{-1} ($P < 0.05$, $n = 7$) in comparison with untreated animals carried out on the same day that gave a response of 89 ± 30 ng mg^{-1} ($n = 8$) to OA challenge.

Figure 3 shows the effect of various drug treatments on the response to OA at 2 h. In this series of experiments, intravesical administration of saline at 2 h in control, OA sensitized rats, produced a PPE response of 31 ± 7 ng mg^{-1} ($n = 6$) while OA instillation in vehicle-treated animals gave a response of 71 ± 11 ng mg^{-1} ($n = 20$). Neither the intravenous administration of the tachykinin NK_2 receptor antagonist MEN 11420 (0.1 μmol kg^{-1}, $n = 8$), although it did inhibit the inflammatory response by about 50%, nor the B_1 bradykinin receptor antagonist B 9858 (0.1 μmol kg^{-1}, $n = 8$) had any significant effect on the response to OA. In animals treated with higher dose of MEN 11420 (1 μmol kg^{-1}), OA gave a PPE response 20% greater than the control response (data not shown). In contrast Hoe 140 (0.1 μmol kg^{-1}, $n = 10$), dexketoprofen (5.3 μmol kg^{-1}, $n = 11$) and SR 140333 (0.1 μmol kg^{-1}, $n = 10$) abolished the effect of OA, ONO 1078 (1 μmol kg^{-1}, $n = 11$) significantly reduced by 77% the PPE response to OA.

In vitro *smooth muscle reactivity*

OA (1 mg ml^{-1}) caused contraction of isolated bladder strips (see Figure 4 for a typical response). The response to OA was immediate and sustained for at least 25 min. On isolated bladder strips from unsensitized animals OA had no constrictor effect ($n = 4$). *In vitro* capsaicin desensitization whilst having no effect on the magnitude of the response to OA (see Table 2) it did suppress the duration of the response (Figures 4 and 5). *In vivo* pretreatment with compound 48/80 (5 mg kg^{-1}, s.c., for 3 consecutive days) resulted in a significant inhibition of the contractile response at all time points ($n = 7$) and *in vitro* capsaicin treatment of bladders taken from these animals resulted in almost complete abolition of the response ($n = 7$, Figure 5). This compound 48/80 treatment regiment was shown to be effective in degranulating mast cells, since *in vitro* compound 48/80 (0.1 mg ml^{-1}) gave a maximum contractile response of $57 \pm 8\%$ ($n = 4$) and $2 \pm 2\%$ ($n = 4$) of the response to NKA (0.1 μM) in control untreated sensitized animals and in animals pretreated with compound 48/80, respectively.

Neither ONO 1078 (1 μM, $n = 8$) nor Hoe 140 (1 μM, $n = 5$) had any effect on the contractile response to OA (Table 2).

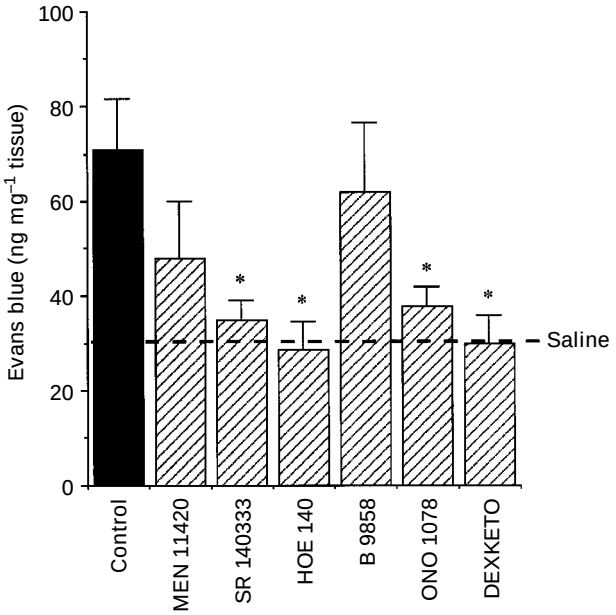

Figure 3 Effect of a range of different selective receptor antagonists and enzyme inhibitor (dexketo for dexketoprofen) administered intravenously, on plasma protein extravasation induced by intravesical ovalbumin challenge (OA 10 mg in 0.3 ml saline) at 2 h time point in the urethane-anaesthetized rat. Dotted line represents the PPE value at 2 h following saline intravesical instillation. Doses and time of administration are showed in Table 1. Values shown are mean $\pm$ s.e.mean of $n = 8-20$ animals. Statistical significance is shown as $^*P < 0.05$, compared with the control values.

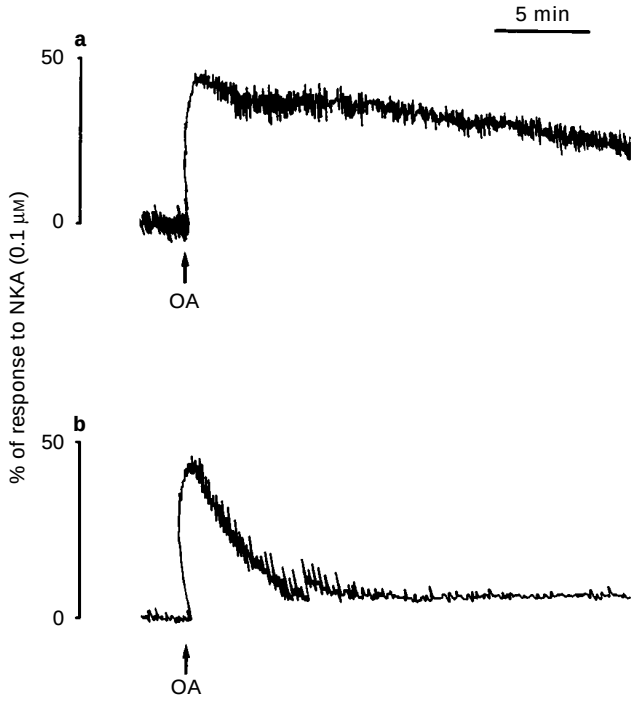

Figure 4 Typical tracings showing the contractile effect of ovalbumin (OA, 1 mg ml^{-1}) on isolated bladder preparations from OA sensitized rats (a) and OA-sensitized rats after *in vitro* capsaicin desensitization (10 μM for 15 min) (b).

Table 2 Maximum responses of ovalbumin (OA) sensitized rat isolated urinary bladder strips to acute challenge with ovalbumin (1 mg ml^{-1}) in the control and drug-treated tissues

Pretreatment	*Contractile response to OA* (% of NKA 0.1 μM)	*Significance (number of animals)*
Control	37.4 ± 3.1	($n = 7$)
Capsaicin (10 μM, 20 min)	32.1 ± 4.2	NS ($n = 7$)
Compound 48/80 (3 day s.c. 5 mg kg^{-1})	22.0 ± 4.8	$P < 0.05$ ($n = 7$)
Capsaicin + compound 48/80	12.7 ± 3.2	$P < 0.01$ ($n = 7$)
ONO 1078 (1 μM, 15 min)	33.5 ± 4.9	NS ($n = 8$)
Hoe 140 (1 μM, 15 min)	37.0 ± 5.4	NS ($n = 5$)

Values shown are mean $\pm$ s.e.mean. Statistical significance was determined by ANOVA followed by Dunnett's test for multiple comparison. NS: not significant.

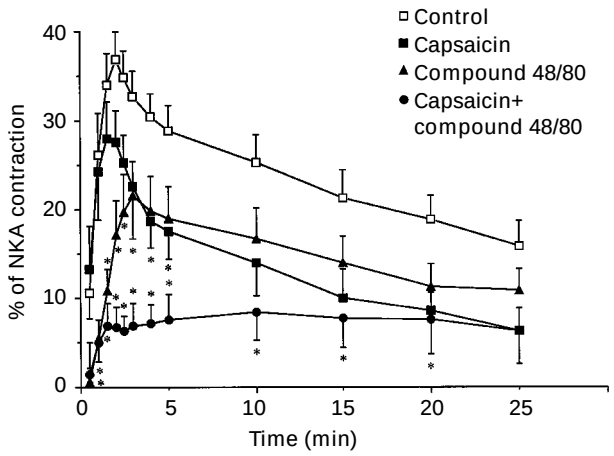

Figure 5 Modulation of ovalbumin-induced contraction of isolated bladder preparations from OA-sensitized rats by capsaicin and compound 48/80. The responses to OA in control untreated tissues, in tissues pretreated with capsaicin (10 μM for 15 min), in tissues taken from animals pretreated chronically with compound 48/80 (5 mg kg^{-1} for three consecutive days), or both treatments combined, are shown. The responses are calculated as % of the maximal contraction to 0.1 μM neurokinin A. Values shown are mean of $n=7$ experiments; vertical lines indicate s.e.mean. Statistical significance is shown as $^*P<0.05$, compared with the respective control values.

Discussion

Interstitial cystitis at present has an unknown aetiology, although there have been some suggestions that this disease may be immunologically based. Models have been established in the guinea-pig to determine whether immediate hypersensitivity allergic reaction in the bladder mimics the characteristics of interstitial cystitis (Christensen *et al*., 1990). We have now established a model of bladder allergy by use of local challenge with OA in OA sensitized rats. We have shown that there is a time-dependent PPE response to OA. Further the observed PPE response is a consequence of mast cell degranulation and activation of capsaicin-sensitive afferent nerves.

The intravesical application of OA caused measurable PPE over the 2–24 h time points with no significant effect at the 0.5 h time point. This may reflect the time required for the antigen to actually cross the urothelium. In other systems where there is no barrier to the passage of the antigen the early response to OA in a sensitized animal is almost immediate. For instance IgE-mediated dermal hypersensitivity responses, as characterized by wheal and flare and due to the degranulation of mast cells (Wasserman, 1990; Galli, 1993), develop rapidly in man reaching a peak response at approximately 10–20 min (Solley *et al*., 1976; Wasserman, 1990). Despite the differences in time of onset of the response, the PPE that occurred in the bladder was significantly attenuated in those animals which had been systemically treated with compound 48/80, indicating as with other systems modelling immediate hypersensitivity that the inflammatory response is mast cell-dependent. The treatment regimen of compound 48/80 used for these studies has previously been shown to be an effective temporary method for the removal of functional mast cells (Eglezos *et al*., 1992) and, indeed, the efficacy of this treatment was verified in the present study by the fact that compound 48/80 (0.1 mg ml^{-1}) had no contractile effect in isolated bladder strips taken from animals subjected to the 3 days degranulation procedure.

The urinary bladder is endowed with a dense sensory innervation which is involved in the control of normal vesicourethral function. However, it is also clear that capsaicin-sensitive afferent nerves play an important role in the induction and perpetuation of certain urinary pathologies. Indeed systemic pretreatment with capsaicin resulted in attenuation of the response to OA over the entire time range, except for the response at 24 h. At this time the PPE response produced by OA administration seems to be independent of an active contribution of capsaicin-sensitive primary afferent neurons (neurogenic inflammation) but may be induced by inflammatory cell accumulation (eosinophils). These results show that the early response to OA in the bladder of sensitized rats involve both C-fibre and mast cell activation. This dependency on intact afferent nerves is similar to several other types of cystitis model demonstrating significant involvement of sensory fibres in the inflammatory component of the response (Maggi, 1995).

In the present study the instillation of saline produced a mild PPE response above baseline at the 4 h time point which is probably attributable to mechanical irritation of the bladder mucosa. A previous study (Giuliani *et al*., 1993) showed that the catheterization required for intravesical instillation into the female rat bladder produced an inflammatory response abolished by the combination of the selective bradykinin B_2 antagonist Hoe 140 and tachykinin NK_1 receptor antagonist CP96345. These findings have been interpreted as the mechanical trauma inducing bradykinin formation in the bladder wall leading to tachykinin release. The 2 h time point was taken for all of the ensuing studies, since the response was significantly attenuated by capsaicin pretreatment, and this was also the time point at which a near-maximal response was seen and the catheterization did not induce an inflammatory response on its own.

Characterization of the sensory component, at the 2 h time point, indicates that this response is a complex process involving an interplay between multiple mediators produced during neurogenic inflammation. The tachykinin NK_1 receptor blocker, SR 140333, significantly inhibited the OA-induced PPE implying that substance P (SP) is a mediator of the PPE response at 2 h. This is in line with studies showing that SR 140333, dose-dependently, blocked PPE responses to local OA challenge or SP application in the skin of sensitized rats (Herbert & Bernat, 1996). In contrast the tachykinin NK_2 receptor blocker MEN 11420 had no effect. In the knee joint it has been demonstrated that blockade of either tachykinin NK_1 or NK_2 receptors alone does not alter basal synovial blood flow. However, co-administration of these antagonists significantly reduced basal perfusion (Ferrell *et al*., 1997). Unlike this study, the combination of the two antagonists had no greater effect than the NK_1 receptor blocker alone (data not shown) suggesting that in the rat urinary bladder co-operation between the two tachykinin receptors does not exist. In addition it is likely that the tachykinin mediating the NK_1 response is SP. Indeed, sensory nerve-derived tachykinins have been significantly implicated in other rat models of cystitis by use of xylene (Giuliani *et al*., 1993) or cyclophosphamide (Ahluwalia *et al*., 1994). Furthermore in the sensitized guinea-pig it has been demonstrated that SP, administered intravesically, has a similar inflammatory effect to that of intravesical OA (Bjorling *et al*., 1994).

As with other rat models of cystitis the allergen-induced plasma extravasation is also sensitive to blockade of bradykinin B_2 receptors, since Hoe 140 produced significant inhibition of the extravasation response whilst blockade of kinin B_1 receptors had no effect. Additionally the cyclo-oxygenase inhibitor, dexketoprofen, also blocked the oedema response. There is previous evidence for both bradykinin and

prostaglandin-induced activation of capsaicin-sensitive sensory nerves (Maggi, 1995). It has been shown that bradykinin-induced contraction of the urinary bladder is associated with prostaglandin production (Maggi *et al*., 1989); furthermore bradykinin-induced depolarization of sensory nerves with consequent neuropeptide release is also sensitive to the cyclo-oxygenase inhibitor, indomethacin (Maggi, 1991). Thus in the present study it is possible that following mast cell degranulation the activation of capsaicin-sensitive afferent nerves, leading to PPE, involves both bradykinin production and consequent prostaglandin release.

Studies in the guinea-pig clearly show that leukotrienes are both released and play a role in bladder smooth muscle reactivity in response to antigen (Lee *et al*., 1995) and in antigen-induced bronchoconstriction in sensitized guinea-pigs (Nakagawa *et al*., 1993). Our results showed that the leukotriene antagonist ONO 1078 suppress the oedema response to OA also in the rat bladder.

The *in vitro* studies, similar to the PPE studies, clearly show the involvement of both mast cell degranulation and sensory nerve activation in the bladder response to antigen. *In vitro* desensitization with capsaicin had no significant effect on the magnitude of the contractile response to OA but did significantly attenuate the duration of the response. In contrast pretreatment of animals with compound 48/80 resulted in a slowly developing contractile response with significant attenuation of the maximum response. Thus from these experiments it appears that mast cell degranulation and consequent release of contractile mediators occurs immediately producing a rapidly developing contraction. This is followed by activation of capsaicin-sensitive afferents and presumably release of neuropeptide that maintain the contractile response over 25 min. Interestingly a combination of the drug treatments almost abolished the response, which could suggest one or two possibilities. Firstly, the IgE recognition occurs not only on mast cells but possibly also on the sensory nerves. However, there is currently no precedent for such a hypothesis. The second possibility is that there was incomplete degranulation of mast cells with the protocol used. Since the application of compound 48/80 ($0.1\ mg\ ml^{-1}$) in isolated bladder preparations, taken from rats subjected to systemic compound 48/80 treatment, did still show a small contraction, this latter possibility may be the case but this warrants further investigation. As with cyclophosphamide-induced cystitis (Ahluwalia *et al*., 1994), the bradykinin B_2 receptor antagonist significantly inhibited the OA-induced PPE, but it had no effect on bladder smooth muscle contraction, demonstrating that whilst bradykinin plays an important role in the oedema component of cystitis it may not be important in mediating the functional changes in smooth muscle contractility. Likewise, blockade of leukotriene receptors with ONO 1078 did not affect the OA-induced contractions in isolated bladder strips, although the leukotriene antagonists suppressed the bladder oedema response to OA.

In conclusion, the present studies clearly show that acute intravesical challenge of OA-sensitized rats causes both plasma extravasation and contraction of bladder smooth muscle, characteristics of interstitial cystitis (Messing, 1987), following activation of capsaicin-sensitive afferent nerves. Importantly, whilst both responses are mast cell- and sensory fibre-dependent, the mechanisms involved in these two distinct, yet related, processes are different. Thus local allergic reaction within the bladder involves the release and activation of multiple inflammatory mediators which act together to produce features characteristic of interstitial cystitis.

A.A. is the recipient of a BHF intermediate Fellowship.

References

AHLUWALIA, A., MAGGI, C.A., SANTICIOLI, P., LECCI, A. & GIULIANI, S. (1994). Characterization of the capsaicin-sensitive component of cyclophosphamide-induced inflammation in the rat urinary bladder. *Br. J. Pharmacol.,* **111,** 1017–1022.

BJORLING, D.E., SABAN, M.R. & SABAN, R. (1994). Neurogenic inflammation of the sensitized guinea-pig bladder. *Mediators of Inflamm.,* **3,** 193–198.

CHRISTENSEN, M.M., KEITH, I., RHODES, P.R., GRAZIANO, F.M., MADSEN, P.O., BRUSKEWITZ, R.C. & SABAN, R. (1990). A guinea-pig model for study of bladder mast cell function: histamine release and smooth muscle contraction. *J. Urol.,* **144,** 1290–1293.

EGLEZOS, A., LECCI, A., SANTICIOLI, P., GIULIANI, S., TRAMONTANA, M., DEL BIANCO, E. & MAGGI, C.A. (1992). Activation of capsaicin-sensitive primary afferents in the rat urinary bladder by compound 48/80: a direct action on sensory nerves? *Arch. Int. Pharmacodyn. Ther.,* **315,** 96–109.

EMONDS-ALT, X., DOUTREMEPUICH, J.D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.P., PROIETTO, V., VAN BROECK, D., SOUBRIE, P., LE FUR, G. & BRELIERE, J.C. (1993). In vitro and in vivo biological activities of SR 140333, a novel potent non-peptide tachykinin NK_1 receptor antagonist. *Eur. J. Pharmacol.,* **250,** 403–413.

EVANS, T.W., ROGERS, D.F., AURSUDKIJ, B., FAN CHUNG, K. & BARNES, P.J. (1988). Inflammatory mediators involved in antigen-induced airway microvascular leakage in guinea-pigs. *Am. Rev. Respir. Dis.,* **138,** 395–399.

FERRELL, W.R., LOCKHART, J.C. & KARIMIAN, S.M. (1997). Tachykinin regulation of basal synovial blood flow. *Br. J. Pharmacol.,* **121,** 29–34.

GALLI, S.J. (1993). New concepts about the mast cell. *New Eng. J. Med.,* **328,** 257–265.

GIULIANI, S., SANTICIOLI, P., LIPPE, I. Th., LECCI, A. & MAGGI, C.A. (1993). Effect of bradykinin and tachykinin receptor antagonists on xylene-induced cystitis in rats. *J. Urol.,* **150,** 1014–1017.

HELLSTROM, H.R., DAVIS, B.K. & SHONNARD, J.W. (1979). Eosinophilic cystitis. *Am. J. Clin. Pathol.,* **72,** 777–784.

HERBERT, J.M. & BERNAT, A. (1996). Effect of SR 140333, a selective NK_1 antagonist, on antigen-induced oedema formation in rat skin. *J. Lipid Mediat. Cell Signal.,* **13,** 223–232.

HOGAN, S.P. & FOSTER, P.S. (1996). Cellular and molecular mechanism involved in the regulation of eosinophil trafficking in vivo. *Med. Res. Rev.,* **16,** 407–432.

HOLLANDER, D.H. (1994). Interstitial cystitis and silk allergy. *Med. Hypoth.,* **43,** 155–156.

KIM, Y.S., LONGHURST, P.A., WEIN, A.J. & LEVIN, R.M. (1991). Effects of sensitisation on female guinea-pig urinary bladder function: in vivo and in vitro studies. *J. Urol.,* **146,** 454–457.

LECCI, A., GIULIANI, S., TRAMONTANA, M., CRISCUOLI, M. & MAGGI, C.A. (1997). MEN 11420, a peptide tachykinin NK_2 receptor antagonist, reduces motor responses induced by the intravesical administration of capsaicin in vivo. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** 182–188.

LEE, J.G., LEVIN, R.M., KRASNOPOLSKY, L., WEIN, A.J. & LONGHURST, P.A. (1995). Effects of the peptide leukotriene receptor antagonist ICI 198,615 on the in vivo and in vitro changes in guinea-pig bladder function which occur after sensitisation with ovalbumin. *J. Urol.,* **154,** 1217–1221.

MAGGI, C.A. (1991). The pharmacology of the efferent function of sensory nerves. *J. Auton. Pharmacol.,* **11,** 173–208.

MAGGI, C.A. (1995). Tachykinins and calcitonin gene-related peptide (CGRP) as cotransmitters released from peripheral endings of sensory nerves. *Prog. Neurobiol.,* **45,** 1–98.

MAGGI, C.A., GEPPETTI, P., SANTICIOLI, P., FRILLI, S., GIULIANI, S., FURIO, M., THEODORSSON, E., FUSCO, B. & MELI, A. (1988). Tachykinin-like immunoreactivity in the mammalian urinary bladder: correlation with the functions of the capsaicin-sensitive sensory nerves. *Neuroscience,* **26,** 233–242.

MAGGI, C.A., PATACCHINI, R., SANTICIOLI, P., GEPPETTI, P., CECCONI, R., GIULIANI, S. & MELI, A. (1989). Multiple mechanisms in the motor responses of the guinea-pig isolated urinary bladder to bradykinin. *Br. J. Pharmacol.,* **98,** 619–629.

MAGGI, C.A., SANTICIOLI, P., DEL BIANCO, E., LECCI, A. & GIULIANI, S. (1993). Evidence for the involvement of bradykinin in chemically-evoked cystitis in anaesthetised rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **347,** 432–437.

MAULEON, D., ARTIGAS, R., GARCIA, M.L. & CARGANICO, G. (1996). Preclinical and clinical development of dexketoprofen. *Drugs,* **52,** 24–46.

MESSING, E.M. (1987). The diagnosis of interstitial cystitis. *Urology, S4,* **29,** 4.

MESSING, E.M. & STAMEY, T.A. (1978). Interstitial cystitis. Early diagnosis, pathology and treatment. *Urology,* **12,** 380–381.

NAKAGAWA, N., OBATA, T., KOBAYASHI, T., OKADA, Y., NAMBU, F., TERAWAKI, T., FURUYA, T., MURYOBAYASHI, K., SAWADA, M. & AISHITA, H. (1993). Effect of a peptide leukotriene receptor antagonist, ONO-1078, on guinea-pig models of asthma. *Eur. J. Pharmacol.,* **235,** 211–219.

SARIA, A. & LUNDBERG, J.M. (1983). Evans blue fluorescence: quantitative and morphological evaluation of vascular permeability in animal tissue. *J. Neurosci. Methods,* **8,** 41–49.

SOLLEY, G.P., GLEICH, G.J., JORDON, R.E. & SCHROETER, A.L. (1976). The late phase of the immediate wheal and flare skin reaction. *J. Clin. Invest.,* **58,** 408–420.

STEWART, J.M., GERA, L., HANSON, W., ZUZACK, J.S., BURKARD, M., MCCULLOUGH, R. & WHALLEY, E.T. (1996). A new generation of bradykinin antagonists. *Immunopharmacology,* **33,** 51–60.

WASSERMAN, S. (1990). Mast cell biology. *J. Allergy. Clin. Immunol.,* **86,** 590–593.

(Received January 12, 1998
Accepted January 28, 1998)